Supplemental Material for: An Assessment of Exposure to Prescribed Estrogens in Drinking Water

Daniel Caldwell,[1] Frank Mastrocco,[2] Edward Nowak,[3] James Johnston,[4] Harry Yekel,[4]

Danielle Pfeiffer,[5] Marilyn Hoyt,[5] Beth M. DuPlessie,[5] Paul D. Anderson[5,6]*

[1]Johnson & Johnson Worldwide Environment, Health, and Safety, New Brunswick, New Jersey, USA; [2]Pfizer Inc, New York, New York, USA; [3]Johnson & Johnson Pharmaceutical Research and Development, Raritan, New Jersey, USA; [4]Wyeth, Madison, New Jersey; [5]AMEC Earth & Environmental, Westford, Massachusetts, USA; [6]Department of Geography and Environment, Boston University, Boston, Massachusetts, USA

* Corresponding author: Paul D. Anderson, AMEC Earth & Environmental, 2 Robbins Road, Westford, MA 01886, USA. Telephone: 978-692-9090. Fax: 978-692-6633. E-mail: paul.anderson@amec.com

Comparison of Estrogen PECs generated by P*h*ATE to Measured Estrogen Concentrations in Drinking Water.

Relatively few studies have analyzed U.S. drinking waters derived from surface water for the presence of estrogens (no data are available for estriol (E3)) and all report non detects. Boyd et al. (2003) analyzed drinking water at a plant in Louisiana and another in Ontario and did not detect estrone (E1) or 17β-estradiol (E2) (method detection limits of 0.3 and 0.1 ng/l, respectively). Jasim et al (2006) also did not detect E1 or E2 (minimum detection limits of 410 and 400 ng/l) in Ontario drinking water. Similarly, McQuillan et al. (2001) did not detect either

E1, E2 or ethinyl estradiol (EE2) (detection limit of 10 ng/l) in finished water from two drinking water treatment plants in New Mexico. More recently, Benotti et al (2009) also reported non-detects for E1, E2 and EE2 in 18 finished drinking water samples (detection limits ranging from 0.2 to 1.0 ng/l). These results are consistent with the PECs from P*h*ATE (Table 2 and Figure 1) in that average mean and low flow PECs are predicted to be less than the detection limit as are the 90th percentile PECs for E2 and EE2. The 90th percentile PEC for E1 (3.06 ng/l) is above the lowest analytical detection limit (Boyd et al. 2003) but that does not indicate an inconsistency where modeled concentrations exceed measured concentrations. Very few drinking water treatment plants have been sampled to date and, thus, the upper and lower percentiles of the drinking water concentration distribution may not be represented in the few available measurements.

Drinking water data are also available from other continents (Adler et al. 2001; Aherne and Briggs 1989; Aherne et al. 1985; Brown et al. 2001; Fawell et al. 2001; Kuch and Ballschmiter 2001; Morteani et al. 2006; Rodriguez-Mozaz et al. 2006; Wen et al. 2006). These researchers also report generally non detectable concentrations at levels that are consistent with the PECs generated by P*h*ATE (Table 2 and Figure 1), though in some cases, detections exceed the maximum PECs (EE2 reported by Adler et al. (2001) and Morteani et al. (2006); E2 and EE2 reported by Kuch and Ballschmiter (2001)). This finding does not mean the PECs generated by P*h*ATE for U.S. drinking waters are low. Rather, they may be indicative of differences in treatment plant removal of estrogens between countries.

In summary, comparison of drinking water PECs developed by P*h*ATE to the few available MECs from the United States indicates the modeled concentrations are consistent with measured

concentrations. These findings parallel those of Anderson et al. (2004) and Hannah et al, in press, for surface water PECs.

Estimating Dietary Exposure

Dietary exposures for a child (ages 1-7) and an adult (ages 16-75) are estimated using published data for concentrations of endogenous estrogens in major foods (Table SM-1) and mean body weights and specific ingestion rates for age and gender groups as published by EPA (U.S. EPA 1997) (Table SM-2). A summary of the estimated dietary intake of endogeneous estrogens from omnivore diet is presented in Table SM-3.

It should be noted that totals for dietary intake of estrogens are likely biased low because of missing data for one or more of the estrogens in a particular food or for dietary intake of other foodstuffs that would contain estrogens but for which concentration data are not available.

Estimating Drinking Water Exposure - Loading of Prescribed Estrogens to POTWs

The mass of prescribed hormone that is excreted and available to enter a POTW is a function of the daily per capita use less the amount assumed to be metabolized (or lost in transit from the point of excretion to the POTW). IMS sales data for the 12 months from March 2007 to February 2008 were used to estimate per capita use of EE2 and the HT and HRT hormones (IMS 2008, Table SM-4).

Estimating Drinking Water Exposure - Human Metabolism of Prescribed Estrogens

Metabolism of prescribed hormones was based upon review of information in the scientific literature. Johnson and Williams (2004) evaluated the fraction of the EE2 dose excreted based on measured values in urine and feces. They estimate the fraction of EE2 excreted in feces is 30%, of which 77% is present as EE2 or 23% of the dose; and the fraction excreted in urine is 27%, including glucoronide and sulfate conjugates. Although Johnson and

Williams (2004) assumed that sulfate conjugates are not de-conjugated, there is some evidence of sulfate de-conjugation in both humans and sewer systems. Therefore, this evaluation, assumes that 50% of the EE2 dose (23% in feces plus 27% in urine) is excreted by patients as EE2 or as O-glucuronide conjugates subject to de-conjugation in POTWs. Sulfate conjugates (a relatively small percentage) are also assumed to regenerate to the active ingredient. Metabolism of the remaining endogenous hormones is summarized in Table SM-4 and is based upon a review of the literature (Adams et al. 1979; Düsterberg et al. 1985; Friel et al. 2005; Johnson and Williams 2004).

Estimating Drinking Water Exposure - Loading of Endogenous Estrogens to POTWs

Excretion data for endogenous estrogens have been reported from several studies over the last 30 years. Excretion of endogenous estrogens is primarily a function of gender, age and pregnancy status, although dietary fiber and race are also factors. Urinary and fecal excretion rate information available in the open literature was summarized for different genders, ages and pregnancy status and an average excretion rate for each group was calculated. Data reported as conjugated estrogens was adjusted to reflect the levels of "free" estrogens. U.S. census data from 2001 were then used to determine the fraction of the U.S. population each of these different groups represent, and total excretion rates were estimated (Table SM-4).

Summary of POTW removal data for Endogenous and Synthetic Estrogens

Estrogen sulfates or glucuronide conjugates present in urine are readily converted to the active free estrogens in sewer systems by E. coli bacteria (Andersen et al. 2003; Baronti et al. 2000). POTW removal data for E1, E2, E3 and EE2 were obtained from peer-reviewed literature sources. Information obtained from each literature source is summarized below, along with the median and average reported values (Tables SM-5 through SM-8). The number of facilities

94 referenced within each source was taken into account when calculating the average and the
95 median values. A "Secondary treatment" POTW was defined as a facility with activated sludge
96 process, while an "advanced secondary treatment" POTW was defined as a facility with
97 activated sludge process coupled with nutrient removal, fixed bed reactors and/or membrane
98 bioreactors. Due to limited available data for the advanced secondary treatment of EE2 and E3,
99 the average and median removal values for both secondary and advanced secondary treatment
100 were calculated using all available data for secondary and advanced secondary treatment. The
101 median removal rate was used to estimate drinking water PECs.
102 Given the absence of information on the removal of estrogens by drinking water treatment
103 systems, this assessment assumes no removal during drinking water treatment. If estrogens are
104 removed during drinking water treatment, the drinking water PECs presented in this paper are
105 overestimates of actual drinking water concentrations.
106 Summary of In-Stream Removal for Endogenous and Synthetic Estrogens
107 Available in-stream removal data for endogenous and synthetic estrogens were obtained from
108 peer-reviewed literature sources. In-stream removal rates employed in this study are
109 summarized in Table SM-9.
110 Adjusting Mass-Based Exposures for Differences in Relative Biological Activity
111 The biological activities of the different estrogens are not equal. To estimate the total
112 estrogenic activity in a particular food or the diet as a whole, these differences in biological
113 activity need to be accounted for. Though a long-held belief exists that receptor binding and
114 potency are related (Korenman 1969), more recent research has shown that the ability of a
115 compound with estrogenic activity to elicit a response varies greatly from one organ, tissue and
116 endpoint to another and is not determined solely by receptor binding affinity (Lundeen et al.

1997; Diamanti-Kandarakis et al. 2009). The other factors that affected the nature and strength of a compound's estrogenic activity are related to three general observations (Dey et al. 2000; Katzenellenbogen and Katzenellenbogen 2002; Komm and Bodine 2001; McDonnell 2000).

First, estrogens can interact with two receptors: the alpha and beta estrogen receptors (ERα and ERβ respectively). Individual compounds differ in their ability to bind to the ERα and ERβ receptors. Moreover, the relative concentration of the two receptors varies between organs and tissues. For example, the prostate epithelium contains only ERβ while the uterus contains primarily ERα (Dey et al. 2000). Second, the activity of the receptors (ERα or ERβ) after binding to a particular estrogen varies, leading to a continuum of potential response rather than a simple "on" response when an estrogen is bound to the receptor and an "off" response when no estrogen is bound (Dey et al. 2000). Third, coregulator proteins can interact with the estrogen-receptor complex and modify its activity. These proteins can enhance (coactivators) or suppress (corepressors) the activity of the estrogen-receptor complex (Katzenellenbogen and Katzenellenbogen 2002) and can depend upon the nature of the estrogenic compound itself. Clearly the combination of all of these factors leads to a diverse range of biological responses following exposure to compounds with estrogenic activity (Diamanti-Kandarakis et al. 2009) and these findings form the basis for the discovery and continued search for and development of Selective Endocrine Response Modulators (SERMs) of which tamoxifen is an example.

Nevertheless, it remains clear that large differences in biological activity do exist across the spectrum of compounds with estrogenic activity. For example, the 2,000 or more ug/day of phytoestrogens in a typical U.S. diet does not cause the same effect as taking an oral contraceptive pill containing 35 ug/day of EE2. Using relative differences in ERα or ERβ binding efficiency is a commonly used and accepted, albeit simple and crude, method to

attempt to account for differences in biological activity of various classes and types of estrogens and compounds with estrogenic activity in environmental risk assessments.

A review of relative binding activity of the various estrogens reveals some general patterns across numerous studies (Bovee et al. 2004; Gutendorf and Westendorf 2001; Safford et al. 2003). E1 and E3 have a lower binding efficiency than E2 using either ERα or ERβ (Table 1). The relative binding of E1 to ERα or ERβ is similar while the relative binding of E3 is about four times more efficient for ERβ than ERα (Table SM-10). Because the differences in ERα and ERβ binding for endogenous estrogens are relatively small, this analysis uses the ERα relative binding efficiency to normalize the estrogenic activity of the different estrogens.

The relative receptor binding activity of E2 and EE2 provide an excellent example of the limitations of simply using receptor binding. Both compounds have similar ERα and ERβ receptor binding efficiencies (Bovee et al. 2004; Gutendorf and Westendorf 2001) yet have very different biological activity. A dose of 625 ug of conjugated estrogens is considered equivalent to 5 - 10 ug of ethinyl estradiol (Goodman et al. 1996). The primary reason for the difference is that a larger fraction of the conjugated estrogens than of ethinyl estradiol is deactivated during first pass metabolism. This difference between E2 and EE2 is not captured by relative binding activity. When normalizing estrogen exposures, so as not to underestimate the potential activity of EE2 and associated MOS, this analysis assumes that EE2 has ten times the biological activity of E2 (Table SM-10). This is likely an overestimate of EE2's biological activity. The EE2 OEL is only three times smaller than the E2 OEL (Johnson & Johnson 2004, 2009). In addition, the NOAELs for EE2 and E2 from two recent male rodent reproductive system studies are within two-fold of each other (Howdeshell et al. 2008; Tyl et al. 2008). Both of these lines of evidence support a relative biological activity adjustment of less than 10 for EE2.

References

Adams WP, Hasegawa J, Johnson RN, Haring RC. 1979. Conjugated estrogens bioequivalence: comparison of four products in post-menopausal women. J Pharm Sci 68(8):986-991.

Adler P, Steger-Hartmann T, Kalbfus W. 2001. Distribution of natural and synthetic estrogenic steroid hormones in water samples from southern and middle Germany. Acta Hydrochim Hydrobiol 29(4):227-241.

Aherne GW, English J, Marks V. 1985. The role of immunoassay in the analysis of microcontaminants in water samples. Ecotoxicol Environ Saf 9(1):79-83.

Aherne GW, Briggs R. 1989. The relevance of the presence of certain synthetic steroids in the aquatic environment. J Pharm Pharmacol 41(10):735-736.

Andersen H, Siegrist H, Halling-Sorenson B, Ternes TA. 2003. Fate of estrogens in a municipal sewage treatment plant. Environ Sci Technol 37(18):4021-4026.

Anderson PD, D'Aco VJ, Shanahan P, Chapra SC, Hayes P, Buzby ME, et al. 2004. Screening analysis of human pharmaceuticals in U.S. surface waters. Environ Sci Technol 38(3):838-849.

Baronti C, Curini R, D'Ascenzo G, Corcia AD, Gentili A, Samperi R. 2000. Monitoring natural and synthetic estrogens at activated sludge treatment plants and in a receiving river water. Environ Sci Technol 34(24):5059-5066.

Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. 2009. Pharmaceutical and endocrine disrupting compounds in U.S. drinking water. Environ Sci Technol 43:597-603.

Bovee TFH, Helsdingen RJR, Rietjans IMCM, Keijer J, Hoogenboom RLAP. 2004. Rapid yeast estrogen bioassays stably expressing human estrogen receptors α and β, and green

flourescent protein: a comparison of different compounds with both receptor types. Steroid Biochem Mol Biol 91(3):99-109.

Boyd GR, Reemtsma H, Grimm DA, Mitra S. 2003. Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. Sci Total Environ 311(1-3):135-149.

Brown L, du Preez JL, Meintjies E. 2001. The qualitative and quantitative evaluation of estrogen and estrogen-mimicking substances in the South African water environment: a 1996-1997 perspective. In: Proceedings from the NGWA, 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, October 9-11, 2001. Westerville, OH:National Ground Water Association.

Carballa M, Omil F, Lema JM, Llompart M, Garcia-Jares C, Rodriguez I, et al. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res 38(12):2918-2926.

D'Ascenzo G, Di Corcia A, Gentili A, Mancini R, Mastropasqua R, Nazzari M, et al. 2003. Fate of natural estrogen conjugates in municipal sewage treatment facilities. Sci Total Environ 302(1-3):199-209.

Dey M, Lyttle CR, Pickar JH. 2000. Recent insights into the varying activity of estrogens. Maturitas 34(suppl 2):S25-S33.

Diamanti-Kandarakis E, Bourguignon J-P, Guidice LC, Hauser R, Prins GS, Soto AM, et al. 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 30(4):293–342.

Düsterberg B, Schmidt-Gollwitzer M, Humpel M. 1985. Pharmokinetics and biotransformation of estradiol valerate in ovariectomized women. Horm Res 21(3):145-154

209 Fawell JK, Sheahan D, James HA, Hurst M, Scott S. 2001. Oestrogens and oestrogenic activity
210 in raw and treated water in Severn Trent Water. Water Res 35(5):1240-1244.
211 Friel PN, Hinchcliffe C, Wright JV. 2005. Hormone replacement with estradiol: conventional
212 oral doses result in excessive exposures to estrone. Altern Med Rev 10(1):36-41.
213 Fritsche S, Steinhart H. 1999. Occurrence of hormonally active compounds in food: a review.
214 Eur Food Res Tech 209(3-4):153-179.
215 Goodman LS, Gilman G, Hardman JG, Limbird Le, Molinoff PB, Ruddon RW, eds. 1996.
216 Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 9th ed. New York,
217 NY:McGraw-Hill.
218 Gutendorf B, Westendorf J. 2001. Comparison of an array of in vitro assays for the assessment
219 of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and
220 xenoestrogens. Toxicology 166(1-2):79-89.
221 Hannah R, D'Aco VJ, Anderson PD, Buzby ME, Caldwell DJ, Cunningham VL, et al. In press.
222 exposure assessment of 17α-ethinyl estradiol in surface waters of the United States and
223 Europe. Environ Toxicol Chem.
224 Hartmann S, Lacorn M, Steinhart H. 1998. Natural occurrence of steroid hormones in food. Food
225 Chem 62(1):7-20.
226 Henricks DM, Gray SL, Hoover JLB. 1983. Residue levels of endogenous estrogens in beef
227 tissues. J Anim Sci 57:247-255.
228 Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE Jr. 2008. Gestational and
229 Lactional Exposure to Ethinyl Estradiol, but not Bisphenol A, Decreases Androgen-
230 Dependent Reproductive Organ Weights and Epididymal Sperm Abundance in the Male
231 Long Evans Hooded Rat. Toxicol Sci 102(2):371-382.

IMS. 2008. IMS Midas Quantum Year End February 2008. IMS Health Inc. Available: http://www1.imshealth.com/web/product/0,3155,64576068_63872702_71228109_71461314,00.html [accessed 9 September 2009].

Jasim SY, Irabelli A, Yang P, Ahmed S, Schweitzer L. 2006. Presence of pharmaceuticals and pesticides in Detroit River water and the effect of ozone on removal. Ozone Sci Eng 28(6):415-423.

Johnson & Johnson. 2004. Monograph, Occupational Exposure Limit for 17β-Estradiol. New Brunswick, NJ:Johnson & Johnson.

Johnson & Johnson. 2009. Monograph, Occupational Exposure Limit for Ethinyl Estradiol. New Brunswick, NJ:Johnson & Johnson.

Johnson AC, Belfroid A, Di Corcia A. 2000. Estimating steroid estrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Sci Total Environ 256(2-3):163-173.

Johnson AC, Williams RJ. 2004. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Env Sci Technol 38(13):3649-3658.

Joss A, Andersen H, Ternes T, Richle PR, Siegrist H. 2004a. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consquences for plant optimization. Environ Sci Technol 38(11):3047-3055.

Joss A, Andersen H, Ternes T, Richle PR, Siegrist H. 2004b. Manuscript appendix: diffusive mass transfer across the boundary layer and inside the floc. Environ Sci Technol Appendix 38(11)1-4.

254 Jürgens MD, Holthaus KIE, Johnson AC, Smith JJL, Hetheridge M, Williams RJ. 2002. The
255 potential for estradiol and ethinylestradiol degradation in English rivers. Environ Toxicol
256 Chem 21(3):480-488.
257 Katzenellenbogen BS, Katzenellenbogen JA. 2002. Defining the "S" in SERMs. Science
258 295:2380-2381.
259 Komm BS, Bodine PVN. 2001. Regulation of bone cell functions by estrogens. In: Osteoporosis
260 2nd ed. (Marcus R, Feldman D, Kelsey J, eds). New York:Elsevier Academic Press 1:305-
261 337.
262 Korenman SG. 1969. Comparative binding affinity of estrogens and its relation to estrogenic
263 potency. Steroids 13(2):163-177.
264 Kuch HM, Ballschmiter K. 2001. Determination of endocrine-disrupting phenolic compounds
265 and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter
266 range. Environ Sci Technol 35(15):3201-3206.
267 Labadie P, Budzinski H. 2005. Determination of steroidal hormone profiles along the Jalle
268 d'Eysines River (near Bordeaux, France). Environ Sci Technol 39(14):5113-5120.
269 Lee YC, Wang LM, Xue YH, Ge NC, Yang XM, Chen GH. 2006. Natural Estrogens in the
270 surface water of Shenzhen and the sewage discharge of Hong Kong. Hum Ecol Risk Assess
271 12(2):301-312.
272 Lin AY-C, Reinhard M. 2005. Photodegradation of common environmental pharmaceuticals and
273 estrogens in river water. Environ Toxicol Chem 24(6):1303-1309.
274 Lishman L, Smyth SA, Sarafin K, Kleywegt S, Toito J, Peart T, et al. 2006. Occurrence and
275 reductions of pharmaceuticals and personal care products and estrogens by municipal
276 wastewater treatment plants in Ontario, Canada. Sci Total Environ 367(2-3):544-558.

Lundeen SG, Carver JM, McKean M-L, Winneker RC. 1997. Characterization of the ovariectomized rat model for the evaluation of estrogen effects on plasma cholesterol Levels. Endocrinology 138:1552-1558.\

McDonnell DP. 2000. Selective estrogen receptor modulators (SERMs): a first step in the development of perfect hormone replacement therapy regimen. J Soc Gynecol Investig 7:S10-S15.

McGarrigle HHG, Lachelin GCL. 1983. Oestrone, oestradiol and oestriol glucosiduronates and sulphates in human puerperal plasma and milk. J Steroid Biochem 18(5):607-611.

McQuillan D, Parker J, Chapman TH, Sherrell K, Mills D. 2001. Drug residues in ambient water: initial surveillance in New Mexico, USA. In: Proceedings from the NGWA, 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, October 9-11, 2001. Westerville, OH:National Ground Water Association.

Morteani G, Moeller P, Fuganti A, Paces T. 2006. Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague (Czech Republic). Environ Geochem Health 28(3):257-264.

Rodriguez-Mozaz S, de Alda MJL, Barcelo D. 2006. Fast and simultaneous monitoring of organic pollutants in a drinking water treatment plant by a multi-analyte biosensor followed by LC-MS validation. Talanta 69(2):377-384.

Safford B, Dickens, A, Halleron N, Briggs D, Carthew P, Baker V. 2003. A model to estimate the oestrogen receptor mediated effects from exposure to soy isoflavones in food. Regul Toxicol Pharmacol 38(2):196-209.

298 Servos MR, Bennie DT, Burnison BK, Jurkovic A, McInnis R, Neheli T, et al. 2005. Distribution
299 of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants.
300 Sci Total Environ 336(1-3)155-170.
301 Solé M, López de Alda MJ, Castillo M, Porte C, Ladegaard-Pedersen K, Barceló D. 2000.
302 Estrogenicity determination in sewage treatment plants and surface waters from the
303 Catalonian area (NE Spain). Environ Sci Technol 34(24):5076-5083.
304 Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken R-D, Servos, M. 1999. Behavior and
305 occurrence of estrogens in municipal sewage treatment plants - I. Investigations in
306 Germany, Canada and Brazil. Sci Total Environ 225(1-2):81-90.
307 Ternes TA, Bonerz M, Herrmann N, Teiser B, Andersen HR. 2007. Irrigation of treated
308 wastewater in Braunschweig, Germany: an option to remove pharmaceauticals and musk
309 fragrances. Chemosphere 66(5):894-904.
310 Tsujioka T, Ito S, Ohga A. 1992. Female sex steroid residues in the tissues of steers treated with
311 progesterone and oestradiol-17β. Res Vet Sci 52(1):105-109.
312 Tyl R, Myers C, Marr M, Castillo N, Veselica MM, Joiner RL, et al. 2008. One-generation
313 reproductive toxicity study of dietary 17β estradiol (E2;CAS No. 50-28-2) in CD-1®
314 (Swiss) mice. Reprod Toxicol 25(2):144-160.
315 U.S. EPA. 1997. U.S. Environmental Protection Agency Exposure Factors Handbook. Available:
316 http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464 [accessed 9 September 2009].
317 USDA. 2002. United States Department of Agriculture, National Agricultural Statistics Service
318 Livestock Slaughter: 2001 Summary. Available:
319 http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2000s/2002/LiveSlauSu-03-01-
320 2002.pdf [accessed 9 September 2009].

321 Wen Y, Zhou B-S, Xu Y, Jin SW, Feng Y-Q. 2006. Analysis of estrogens in environmental
322 waters using polymer monolith in-polyether ether ketone tube solid-phase microextraction
323 combined with high performance liquid chromatography. J Chromatog A 1133(1-2):21-28.
324 Williams RJ, Johnson AC, Smith JJL, Kanda R. 2003. Steroid estrogens profiles along river
325 stretches arising from sewage treatment works discharges. Environ Sci Technol
326 37(9):1744-1750.
327 Wolford ST, Argoudelis CJ. 1979. Measurement of estrogens in cow's milk, human milk and
328 diary products. J Dairy Sci 62:1458-1463.
329 Zuehlke S, Duennbier U, Lesjean B, Gnirss R, Buisson H. 2006. Long-term comparison of trace
330 organics removal performances between conventional and membrane activated sludge
331 processes. Water Environ Res 78(13):2480-2486.

Table SM-1. Concentration of Endogeneous Estrogens in Foods

Food Consumed	17β Estradiol ng/g	Estrone ng/g	Estriol ng/g	Reference
Cream	0.015	0.26		Hartmann et al. 1998; Fritsche and Steinhart 1999
Butter	0.043	1.04	0.042	Hartmann et al. 1998
Beef	0.015[a]	0.012[a]		Henricks et al. 1983; Tsujioka et al. 1992; USDA 2002
Beef fat	0.018[a]	0.023[a]		Henricks et al. 1983; Tsujioka et al. 1992
Milk	0.055	0.07	0.016	Hartmann et al. 1998; Safford et al. 2003
Cheese	0.02[b]	0.017[b]		Hartmann et al. 1998
Eggs	0.11	0.535		Hartmann et al. 1998
Chicken meat	0.02			Hartmann et al. 1998
Pork muscle	0.045[c]	0.055[c]		Fritsche and Steinhart 1999
Pork fat	0.04[c]	0.04[c]		Fritsche and Steinhart 1999
Pork liver	0.15[c]	0.24[c]		Fritsche and Steinhart 1999
Olive oil		0.02		Hartmann et al. 1998
Ice cream	0.055	0.07	0.016	Hartmann et al. 1998
Dry curd cottage cheese	0.011	0.037	0.016	Hartmann et al. 1998
Nonfat dry milk	0.0013	0.0093	0.0013	Hartmann et al. 1998
Breast milk	0.059[d]	0.124[d]	0.049[d]	McGarrigle and Lachelin 1983; Hartmann et al. 1998; Safford et al. 2003

[a] Weighted average for implant-treated steers and heifers based on USDA livestock slaughter figures

[b] Average for 4 types

[c] Weighted average for castrated males and females based on USDA slaughter figures

[d] Weighted average over 6 months assuming one week at post-natal level (McGarrigle and Lachelin 1983), remainder of weeks at whole milk level (Hartmann et al. 1998; Safford et al. 2003).

Table SM-2. Age-Specific Food Ingestion Rates (g/kgbw-day) used to Calculate Dietary Intake (kg)

Food Consumed	Child (ages 1-7)	Adult (Ages 16-75)
Buttermilk [a, l]	0.25	0.059
Half and Half [a]	0.23	0.054
Cream[a]	0.12	0.028
Butter[a]	0.31	0.072
Beef[b]	1.33	0.71
Milk (total fluid)[c, l]	23.43	3.33
Cheese[d, l]	0.53	0.24
Eggs[e]	0.64	0.24
Chicken meat[f]	1.04	0.50
Pork muscle[g]	0.44	0.22
Pork fat[h, l]	0.51	0.190
Pork liver[i]	0.0048	0.0048
Rice[j]	0.69	0.29
Cooking oil[k]	0.88	0.37
Ice cream[a]	2.19	0.51
Dry curd cottage cheese[a]	0.10	0.022
Nonfat dry milk[a]	0.19	0.04

[a] Per capita estimate (g/day) from Table 11-20 of EPA's 1997 Exposure Factors Handbook, adjusted by average age-specific male/female body weight.
[b] Age-specific per capita estimate from Table 11-3 of EPA's 1997 Exposure Factors Handbook
[c] Age-specific per capita estimate for Total Milk (g/day) from Table 11-12 of EPA's 1997 Exposure Factors Handbook, adjusted by average male/female body weight.
[d] Age-specific mean intake rate (g/day) from Table 11-12 of EPA's 1997 Exposure Factors Handbook, adjusted by average male/female body weight.
[e] Age-specific intakes from Table 11-7 of EPA's 1997 Exposure Factors Handbook
[f] Age-specific per capita intake from Table 11-5 of EPA's 1997 Exposure Factors Handbook
[g] Age-specific per capita intake from Table 11-4 of EPA's 1997 Exposure Factors Handbook
[h] Estimated consumption (g dry weight/day) from Table 11-18 of EPA's 1997 Exposure Factors Handbook, adjusted by average male/female body weight.
[i] Mean per capita estimates from Table 11-9 of EPA's 197 Exposure Factors Handbook
[j] Age-specific per capita intake from Table 12-8 of EPA's 1997 Exposure Factors Handbook
[k] Assumed intake of 1 T/day for 1-6 years and 2 T for >6 years, based on professional judgment.
[l] 50th percentile body weight reported in Tables 7-2 and 7-3 of EPA's Exposure Factors Handbook. Average is average of 50th %ile body weights for males and females. Male 18.1 (child) 78.04 (adult), Female 17.4 (child) 65.76 (adult), Average 17.8 (child) 71.90 (adult)

Table SM-3. Estimated Dietary Intake of Endogeneous Estrogens from Omnivore diet (mg/day)

	Estrogen Intake (mg/day)	
	Male	Female
Children (ages 1-7)		
Total Intake	8.1E-05	8.1E-05
	Percent of Total	
Meat	3.1%	3.1%
Dairy	87.5%	87.5%
Eggs	9.0%	9.0%
Vegetables	0.4%	0.4%
Adults (ages 16-75)		
Total Intake	6.9E-05	5.8E-05
	Percent of Total	
Meat	7.8%	7.8%
Dairy	73.5%	73.5%
Eggs	17.8%	17.8%
Vegetables	0.2%	0.8%

Table SM-4. Summary of the Mass of Estrogens Excreted in the U.S. and Assumed to be Discharged to POTWs

Compound	Volume Sold in U.S. from 3/07 to 2/08[a] (Kg)	Metabolic Loss (%)	Total Excretion (Kg/yr)[b]
Synthetic Estrogens			
Ethinyl estradiol	82.4	50[c]	41.2
HRT Estrogens			
17β Estradiol[d]	508.6	60	152.6 as Estrone, 50.9 as Estradiol
Estrogenic substances, conjugated[e]	536.7	80	95.5 as Estrone[f], 20.4 as Estradiol[f]
Estrogenic substances, esterified[e]	93.8	80	16.8 as Estrone[f]
Estropipate	36.4	80	7.3 as Estrone[f]
Estriol	7.2	0[g]	7.2
Estrone	1.1	80[h]	0.2
Endogenous Estrogens from the Diet and Naturally Produced[i]			
17β Estradiol	N/A	N/A	631
Estrone	N/A	N/A	1030
Estriol	N/A	N/A	8135

[a] Source: IMS (2008). March 2007 to February 2008 data.
[b] Volume Sold and metabolic loss data are used to calculate excretion data.
[c] See text
[d] Metabolism and excretion data obtained from Friel et al. 2005. 75% of estradiol from HRT is excreted as estrone, while 25% is excreted as estradiol.
[e] The mass of conjugated and esterified estrogenic substances has not been adjusted to reflect the free estrogen content of the formulation.
[f] Metabolism data obtained from Adams et al. (1979). 19% of overall excreted "Estrogenic substances, conjugated" is excreted as 17β Estradiol, while 81% is excreted as Estrone (Adams et al. 1979). 100% of Estropipate and "Estrogenic substances, esterified" are assumed to be excreted as estrone.
[g] No information on metabolic loss of estriol was found; therefore, no loss is assumed
[h] The metabolic loss of estrone is assumed to be equal to the metabolic loss of "Estrogenic substances, esterified"
[i] Derivation of Total Excretion mass of natural and dietary endogenous estrogens is presented in Table SM-4.

Table SM-5. Summary of Estrone Removal by Waste Water Treatment Plants

Estrone Secondary Treatment			Estrone Advanced Secondary Treatment		
Percent Removal	Number of Facilities	Reference	Percent Removal	Number of Facilities	Reference
-83.3	1	Carballa et al. 2004	-54.8	1	Servos et al. 2005
3	1	Lishman et al. 2006	-45.8	1	Servos et al. 2005
9	1	Baronti et al. 2000	49	1	Joss et al. 2004a; Joss et al. 2004b
18	1	Baronti et al. 2000	72.7	1	Servos et al. 2005
50	1	Lishman et al. 2006	76.7	1	Servos et al. 2005
61	6	D'Ascenzo et al. 2003	82.1	1	Servos et al. 2005
64	1	Baronti et al. 2000	85.4	1	Servos et al. 2005
69.5	1	Johnson et al. 2000	90	1	Joss et al. 2004a; Joss et al. 2004b
80	1	Lishman et al. 2006	95.1	1	Servos et al. 2005
80.6	1	Servos et al. 2005	96	1	Ternes et al. 2007
82	1	Johnson et al. 2000	96	1	Joss et al. 2004a; Joss et al. 2004b
82	1	Lishman et al. 2006	96	1	Joss et al. 2004a; Joss et al. 2004b
83	1	Ternes et al. 1999	99	1	Andersen et al. 2003
83	1	Lishman et al. 2006	99	1	Joss et al. 2004a; Joss et al. 2004b
84	1	Baronti et al. 2000			
86	1	Baronti et al. 2000			
94	1	Baronti et al. 2000			
95.1	1	Servos et al. 2005			
96	1	Johnson et al. 2000			
60.1	Mean		66.9	Mean	
66.8	Median		87.7	Median	

Table SM-6. Summary of 17β Estradiol Removal by Waste Water Treatment Plants

17β Estradiol Secondary Treatment			17β Estradiol Advanced Secondary Treatment		
Percent Removal	Number of Facilities	Reference	Percent Removal	Number of Facilities	Reference
56	1	Lee et al. 2006	39.5	1	Servos et al. 2005
65	1	Carballa et al. 2004	64	1	Ternes et al. 1999
76	1	Baronti et al. 2000	75.9	1	Servos et al. 2005
80	1	Lee et al. 2006	88	1	Joss et al. 2004a; Joss et al. 2004b
84	1	Baronti et al. 2000	92.7	1	Servos et al. 2005
84.5	1	Johnson et al. 2000	94.7	1	Servos et al. 2005
85	6	D'Ascenzo et al. 2003	95	1	Joss et al. 2004a; Joss et al. 2004b
88	1	Baronti et al. 2000	96	1	Ternes et al. 1999
89	1	Baronti et al. 2000	96.8	1	Servos et al. 2005
92	1	Baronti et al. 2000	97	1	Joss et al. 2004a; Joss et al. 2004b
92	1	Baronti et al. 2000	98	1	Joss et al. 2004a; Joss et al. 2004b
96	1	Johnson et al. 2000	98	1	Joss et al. 2004a; Joss et al. 2004b
96.1	1	Servos et al. 2005	98.2	1	Servos et al. 2005
97.1	1	Servos et al. 2005	98.3	1	Servos et al. 2005
98	1	Johnson et al. 2000			
99.9	1	Ternes et al. 1999			
85.9	Mean		88.0	Mean	
85.0	Median		95.5	Median	

Table SM-7. Summary of Estriol Removal by Waste Water Treatment Plants

Estriol Secondary Treatment

Percent Removal	Number of Facilities	Reference
80.8	1	Solé et al. 2000
81	1	Solé et al. 2000
84	1	Baronti et al. 2000
94	1	Baronti et al. 2000
97	1	Baronti et al. 2000
97	6	D'Ascenzo et al. 2003
98	1	Baronti et al. 2000
99	1	Baronti et al. 2000
99	1	Baronti et al. 2000
93.9	Mean	
97.0	Median	

Table SM-8. Summary of Ethinyl Estradiol Removal by Waste Water Treatment Plants

Ethinyl Estradiol Secondary Treatment			Ethinyl Estradiol Advanced Secondary Treatment		
Percent Removal	Number of Facilities	Reference	Percent Removal	Number of Facilities	Reference
78	1	Ternes et al. 1999	67	1	Ternes et al. 1999
83	1	Baronti et al. 2000	69	1	Joss et al. 2004a; Joss et al. 2004b
84	1	Baronti et al. 2000	71	1	Joss et al. 2004a; Joss et al. 2004b
84.5	1	Johnson et al. 2000	72.5	1	Zuehlke et al. 2006
85	1	Baronti et al. 2000	75	1	Joss et al. 2004a; Joss et al. 2004b
86	1	Baronti et al. 2000	82.8	1	Zuehlke et al. 2006
87	1	Baronti et al. 2000	85	1	Zuehlke et al. 2006
87	1	Baronti et al. 2000	93	1	Andersen et al. 2003
			94	1	Joss et al. 2004a; Joss et al. 2004b
81	Mean of Secondary and Advanced Treatment				
84	Median of Secondary and Advanced Treatment				

Table SM-9. Summary of Instream Removal Summary

Compound	Half-Life in Rivers (days)[a]	In-Stream Decay (l/d)[a]	Reference
Estrone	2.3	0.3	Jürgens et al. 2002; Labadie et al. 2005; Lin and Reinhard 2005; Williams et al. 2003
17β Estradiol	2.3	0.3	Jürgens et al. 2002; Lin and Reinhard 2005
Estriol	0.12	5.7	Lin and Reinhard 2005
Ethinyl estradiol	9.9	0.07	Jürgens et al. 2002; Lin and Reinhard 2005
Estropipate[b]	2.3	0.3	

[a] In-stream removal P*h*ATE inputs are medians of the range of values reported in the cited literature.

[b] All estropipate values were assumed to be equal to estrone values.

571 Table SM-10. Relative Binding Efficiency

Compound	ERα potency relative to Estradiol	ERβ potency relative to Estradiol
17β Estradiol	1	1
Estrone[a]	0.1035	0.0825
Estriol[a]	0.0375	0.1325
Ethinyl estradiol[b]	10	10

[a] Potency values represent midpoint of values obtained from Gutendorf and Westendorf (2001) and Bovee et al. (2004).

[b] Ethinyl estradiol was assumed to be 10 times more potent than 17β Estradiol. See text for explanation.